TAXONOMIC EVALUATION OF JASPLAKINOLIDE-CONTAINING SPONGES OF THE FAMILY COPPATIIDAE

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Much interest has been generated by the isolation of jasplakinolide (or jaspamide), a novel cyclodepsipeptide originating from a variety of marine sponges, but there have been taxonomic problems in assigning specimens from the South Pacific and Indo-Pacific, possessing a similar spicule composition of oxeas and euasters and sharing parallel chemical profiles. From a comparative study of museum types and recent collections of jasplakinolide-containing sponges in the family Coppatiidae, skeletal and external morphology indicate that only one genus (*Jaspis* Gray) and five species are valid (*J. splendens* (de Laubenfels), *J. digonoxea* (de Laubenfels), *J. johnstoni* (Schmidt), *J. serpentina* Wilson, and a *Jaspis* sp. probably new to science), with the genera *Dorypleres* Sollas and *Zaplethea* de Laubenfels relegated into synonymy. ❒ *Porifera, jasplakinolide, cyclodepsipeptide, Coppatiidae, Jaspis, Dorypleres, Zaplethea.*

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Jasplakinolide (jaspamide) represents a structurally novel compound which has been the focus of several natural products and synthetic studies (Zabriskie et al., 1986; Crews et al., 1986; Braekman et al., 1987; Grieco et al., 1988; Rao et al., 1993; Imaeda et al., 1994). The anticancer activity identified in the NCI 60 cell assay promoted this marine secondary metabolite as a potential therapeutic lead. Of particular value is jasplakinolide's biological profile against PC-3 (human prostate cancer) cells. Although several synthetic derivatives have been examined, jasplakinolide proves to be the most potent agent against cancer models. Mechanism based studies have shown this compound to be a specific actin inhibitor which stabilises microfilaments (Senderowicz et al., 1995). Its potential for drug development may therefore be hindered by its inherent toxicity. However, as a tool for study of the cytoskeleton and the numerous processes mediated by actin in eukaryotes, jasplakinolide provides unique qualities.

Three genera have been used to refer to jasplakinolide-containing sponges, all very closely related morphologically and chemically: *Jaspis* Gray, 1867, *Dorypleres* Sollas, 1888 and *Zaplethea* de Laubenfels, 1950. *Jaspis*, with type species *Vioa johnstoni* Schmidt, 1862, was originally described as having two spicule types: fusiform and stellate (Dendy, 1916), but now contains several disparate species justifying its division into at least two separate genera (Boury Esnault, 1973; Hajdu & Van Soest, 1992). Currently species with oxeas, a single category of euasters, and a confused choanosomal arrangement are included in *Jaspis* (Bergquist, 1968; Wiedenmeyer, 1989). *Dorypleres* contains species like *Jaspis* that have more than one category of asters, but the genus has been used by few authors. Topsent (1904) synonomised *Dorypleres* with *Jaspis*, an action subsequently reversed by de Laubenfels (1954), and reversed again by Bergquist (1968) on the basis that the type species, *D. dendyi* Sollas, lacked two categories of asters. *Zaplethea* includes species with oxeas and euasters, in which the oxeas were characteristically bent (twice-bent), but only one species, *Z. digonoxea* de Laubenfels, 1950, was assigned to it. A report of a jasplakinolide producing sponge from Laing Island (Madang, PNG) refers to *Z. digonoxea* as a synonym of *Jaspis johnstoni* Schmidt, 1862 (Braekman, 1987), implicitly merging the two genera.

At least four taxonomic names have been used for thick encrusting jasplakinolide-containing sponges in the South Pacific and Indo-Pacific with a skeleton of oxeas and euasters: *Jaspis* sp. (Zabriske, 1986; Crews, 1986), *Jaspis johnstoni*, *Zaplethea digonoxea* (Braekman, 1987), and *Dorypleres splendens* (Schmitz and Kelly-Borges, pers. comm.). The present study aims to determine the appropriate generic assignment of these species and their relative conspecificity. Two other species (*J. serpentina* Wilson, 1925, and an unidentified species referred to here as *Jaspis* sp. 2), have similar spiculation and external morphology to the jasplakinolide-containing species, and are included here as a comparison to these species.

TABLE 1. List of voucher and type specimens examined (*=specimen known to contain jasplakinolide).

Reference/institution	Material	Locality
USNM		
USNM 23037	*Dorypleres splendens* de Laubenfels 1954	Caroline Islands, Ponape
USNM 21270	*Jaspis serpentina* Wilson 1925	Philippines
USNM 22746	*Zaplethea digonoxea* de Laubenfels 1950	Oahu, Hawaii
MHNG		
LMJG 15256/0	*Vioa johnstoni* Schmidt 1862	Sebenico, Adriatic
LMJG 15257/0	*Vioa johnstoni* Schmidt 1862	Sebenico, Adriatic
LMJG 15258/0	*Vioa johnstoni* Schmidt 1862	Sebenico, Adriatic
SME		
SME E104	*Zaplethea digonoxea diastra* Vacelet & Vasseur 1976	Tuléar, Madagascar
SME Tu 120	*Jaspis cf. johnstoni*	Tuléar, Madagascar
	**Jaspis johnstoni* (*Z. digonoxea*) Braekman 1987	Laing Island, Papua New Guinea
UO		
35-T-93	**Dorypleres splendens*	Palau
UCSC		
91601	**Jaspis* sp.1	Walindi, Papua New Guinea
91629	*Jaspis* sp. 2	Walindi, Papua New Guinea
92102	**Jaspis* sp. 1	Pacific Harbour, Fiji
92204	**Jaspis* sp. 1	Tioman Island, Malaysia
97238	**Jaspis* sp. 1	Madang, Papua New Guinea
92402	**Jaspis* sp. 1	Bali, Indonesia
92405	*Jaspis* sp. 2	Bali, Indonesia
94541	**Jaspis* sp. 1	N Sulawesi, Indonesia
95077	**Jaspis* sp. 1	Milne Bay, Papua New Guinea
96555	**Jaspis* sp. 1	N Sulawesi, Indonesia
96591	*Jaspis* sp. 2	N Sulawesi, Indonesia

MATERIALS AND METHODS

Specimens were collected from various localities in the South Pacific and Indo-Pacific. All samples were collected using SCUBA at approximate depths of 10-25m. Sponges were preserved in 3.7% formalin for one week, and then transferred and stored in 70% ethanol. Morphological characterisation was made from thick sections (Permount embedded) and spicule preparations of each specimen. Spicule sizes represent mean and range values (minimum and maximum) of the spicule length and width for the oxeas, ray length and width for oxyasters I and II and total diameter for oxyasters III. 15 spicules were measured per spicule type. Special attention was given to surface details in order to assess whether this was a valid taxonomic character for *Dorypleres splendens* in particular.

Specimens examined are listed in Table 1. These were acquired from the United States National Museum (USNM), the Landes-Museum Joanneum of Graz (on loan to the Museum d'Histoire Naturelle Genève (MHNG)), the Station Marine d'Endoume, Centre d'Oceanologie de Marseille (SME), the University of Oklahoma (UO) and the University of California, Santa Cruz (UCSC).

RESULTS AND DISCUSSION

Of the material examined (Table 1) five species were differentiated on the basis of their external morphology and skeletal structure (Table 2). In all this material there is great similarity in skeletal composition and arrangement (Figs 1-6). All species possess oxeas and euasters, a confused choanosomal skeletal arrangement and paratangential arrangement of small spicules at the surface. The external morphology of *Jaspis* sp. 1, (UCSC collections) *J. johnstoni/Z. digonoxea* (Braekman, 1987) and *D. splendens* (both USNM 23037 and UO 35-T-93) is very similar, consisting of an encrusting growth form (2-4cm thick) with oscules on lobate protrusions (1-2cm high). The texture is dry and crumbly and the sponges easily torn. The dry voucher of *J. serpentina* (USNM 21270) is similar except that it appears to have grown away from the substrate, anchored by a stalk (Fig. 3A). External morphology of voucher specimens *Z. digonoxea* (USNM 22746) and *Vioa johnstoni* (LMJG 15258/0, 15256/0) are quite different from the other species described above: they are much more delicate and thinly encrusting (1-3mm thick).

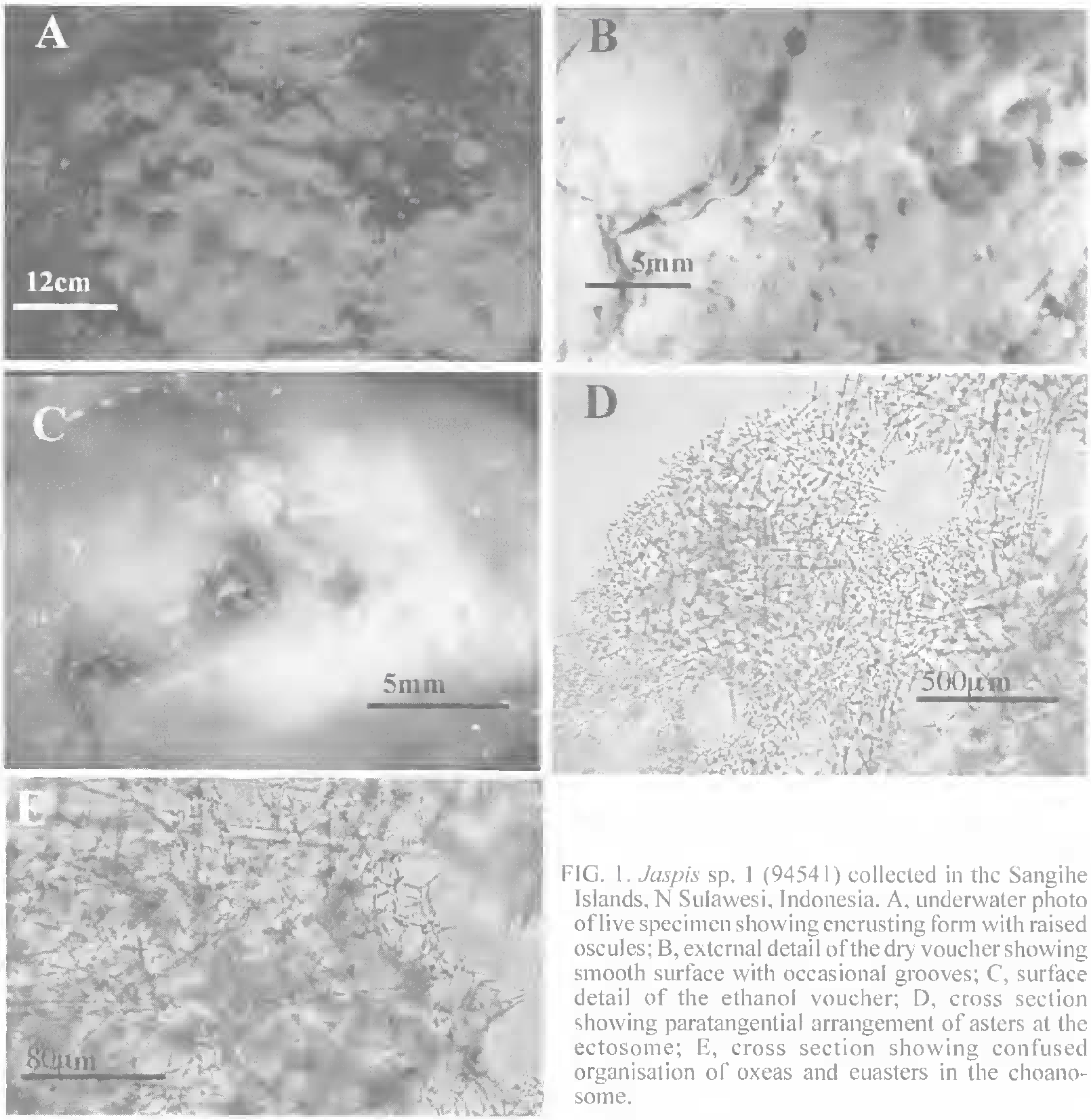

FIG. 1. *Jaspis* sp. 1 (94541) collected in the Sangihe Islands, N Sulawesi, Indonesia. A, underwater photo of live specimen showing encrusting form with raised oscules; B, external detail of the dry voucher showing smooth surface with occasional grooves; C, surface detail of the ethanol voucher; D, cross section showing paratangential arrangement of asters at the ectosome; E, cross section showing confused organisation of oxeas and euasters in the choanosome.

We propose to synonymise all these species under the senior generic name *Jaspis* Gray. The twice-bent oxeas of *Z. digonoxea* de Laubenfels, 1950, are interpreted here as a diagnostic character at the species level only, similar to the serpentine rhabds of *J. serpentina* (Fig. 3B). The proposed assignment of the studied species are: *J. splendens* (de Laubenfels, 1954; Figs 1, 4); *J. digonoxea* (de Laubenfels, 1950; Fig. 5); *J. johnstoni* (Schmidt, 1862; Fig. 6); *J. serpentina* Wilson, 1925, (Fig. 3) and *Jaspis* sp. 2 (Fig. 2), which is probably new to science.

All of the jasplakinolide-containing sponges studied here (Table 1) were found to be conspecific. *Jaspis splendens* (de Laubenfels, 1954) is the senior-most available name for these specimens. The species description requires emendation to de-emphasise the significance of certain surface characteristics given importance by de Laubenfels (1954): conules or protruberances occur as a rather uncommon feature both in the type (Fig. 4B) as well as in more recent collections (Fig. 1B,C).

Figure 2 shows an additional *Jaspis* sp., which although very similar to the others, does not

TABLE 2. Skeletal analysis of specimens of *Jaspis* mentioned in this paper. Measurements given in micrometres as mean (range) of lengths and widths or diameter. 1 = in some specimens this category represents chiasters; 2 = this sample also has serpentine rhabds, 1625 (1550-1700) × 30 (20-40)μm.

Specimen	Reference #	Large oxeas	Small oxeas	Oxyasters I	Oxyasters II	Oxyasters III[1]
Jaspis sp. 1	Crews 92102	741 (530-900) H 8 (5-10)	170 (100-275) H 4 (3-5)	19 (15-30) H 3 (1-4)	16 (13-20)	11 (8-15)
Jaspis sp. 1	Crews 94541	686 (550-780) H 9 (8-10)	130 (88-198) H 3 (2-4)	22 (18-28) H 3 (2-5)	21 (15-28)	8 (5-10)
Jaspis sp. 1	Crews 95077	675 (590-750) H 10 (10)	1222 (80-188) H 3 (2-4)	22 (15-25) H 3 (3)	18 (13-23)	8 (5-10)
Jaspis sp. 1	Crews 96555	637 (520-700) H 10 (10)	120 (78-168) H 3 (2-4)	20 (15-25) H 3 (2-3)	20 (15-30)	10 (8-13)
Jaspis sp. 1	Crews 97238	844 (710-970) H 14 (8-20)	113 (78-160) H 3 (2-5)	23 (18-30) H 3 (3-4)	17 (13-23)	10 (8-13)
Jaspis sp. 2	Crews 96591	777 (650-900) H 11 (10-15)	115 (88-130) H 3 (2-3)	21 (18-28) H 2 (2-3)	19 (15-28)	11 (8-13)
Jaspis sp. 2	Crews 91629	859 (820-920) H 20 (15-25)	156 (105-218) H 5 (3-8)	23 (15-28) H 4 (3-5)	17 (13-20)	10 (8-13)
Dorypleres splendens	Schmitz 35-T-93	665 (600-730) H 9 (7-10)	121 (83-175) H 3 (3-4)	17 (10-23) H 3 (2-3)	13 (8-20)	11 (8-15)
Dorypleres splendens	USNM23037	631 (580-720) H 9 (5-13)	124 (108-175) H 3 (3-5)	19 (13-23) H 3 (2-4)	18 (13-25)	10 (8-13)
Jaspis johnstoni (*Z. digonoxea*)	Braekman, 1987	651 (580-740) H 9 (8-10)	119 (88-163) H 3 (3-4)	18 (13-25) H 2 (2-3)	20 (15-25)	10 (8-13)
Jaspis cf. johnstoni	Vacelet Tu 120	548 (370-920) H 7 (3-15)	98 (73-128) H 3 (2-3)	none	22 (18-30)	11 (8-15)
Zaplethea digonoxea diastra	Vacelet E104	643 (250-1070) H 10 (5-15)	112 (80-148) H 3 (2-4)	none	24 (18-30)	11 (8-15)
Zaplethea digonoxea	USNM22746	751 (560-980) H 10 (5-13)	354 (220-540) H 4 (3-5)	none	21 (15-28)	10 (8-15)
Vioa johnstoni	LMJG15257/0	444 (300-700) H 8 (6-12)	96 (80-110) H 4 (3-5)	none	11 (8-20)	none
Jaspis serpentina	USNM21270[2]	225 (150-350) H 6 (5-8)	81 (63-113) H 3 (2-4)	none	13 (8-20) H 3 (2-5)	8 (6-10)

contain jasplakinolide and differs in external morphology and growth form: it is generally found growing away from the substrate in an encrusting to fan-like form. The topside is very similar to *J. splendens* shown in Fig. 1, but the reverse side is dominated by regularly spaced holes (Fig. 2B). Based on these differences, we suggest that this species is also probably new to science.

CONCLUSIONS

Comparison of type and recently collected material previously assigned to three genera (*Dorypleres splendens*, *Jaspis johnstoni*, *Zaplethea digonoxea*, *Jaspis serpentina*, *Jaspis* sp.) are all referred to *Jaspis*. All *Jaspis*-like species reported in the literature to contain jasplakinolide were examined and referred to *J. splendens* (de Laubenfels), and it is probable that jasplakinolide-containing sponges belong to this species.

ACKNOWLEDGEMENTS

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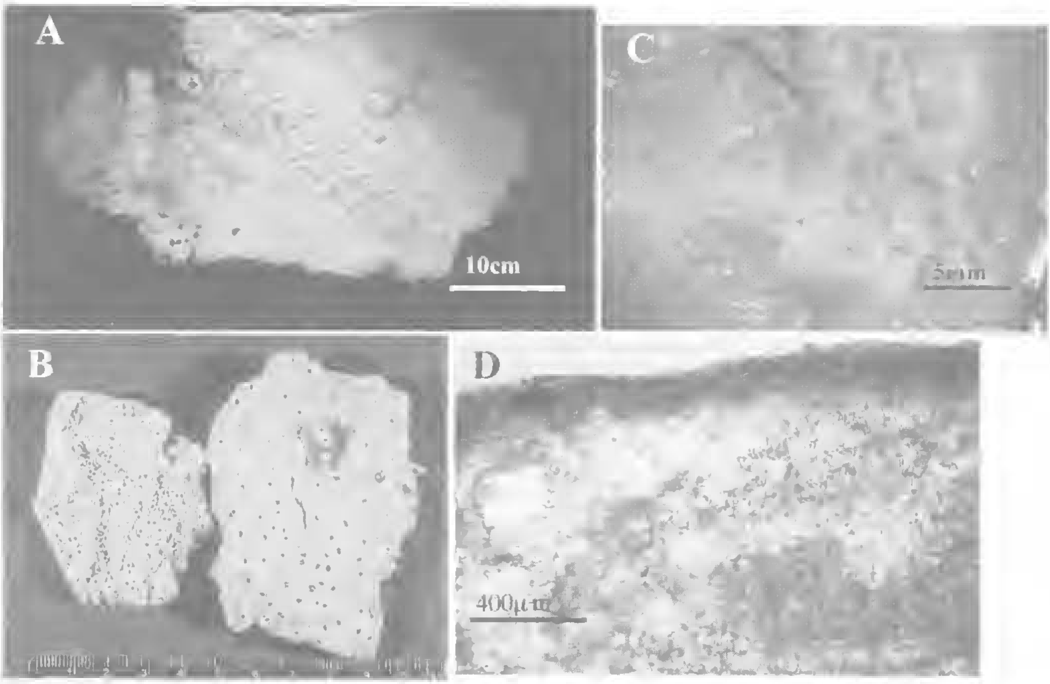

FIG. 2. *Jaspis* sp. 2 (96591) collected at Tifore Island, N Sulawesi, Indonesia. A, underwater photo of live specimen showing encrusting to fan-like morphology; B, external morphology of the dry voucher, topside (left) and underside (right) (scale in cm); C, surface detail of the ethanol voucher; D, cross section showing the concentration of asters at the ectosome and confused organisation of the choanosome.

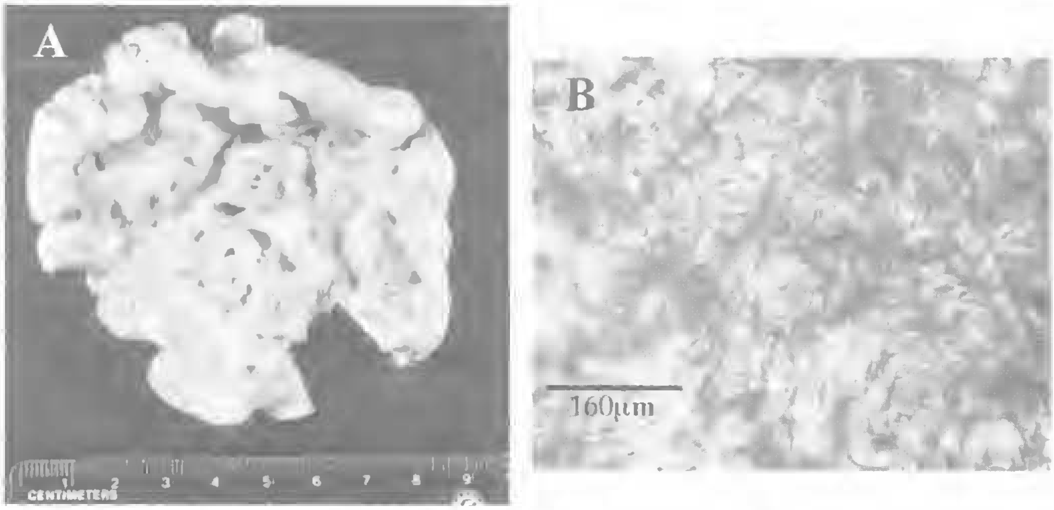

FIG. 3. *Jaspis serpentina* Wilson 1925 (USNM21270). A, dry voucher showing raised oscules and smooth surface; B, cross section of choanosome, dominated by serpentine rhabds.

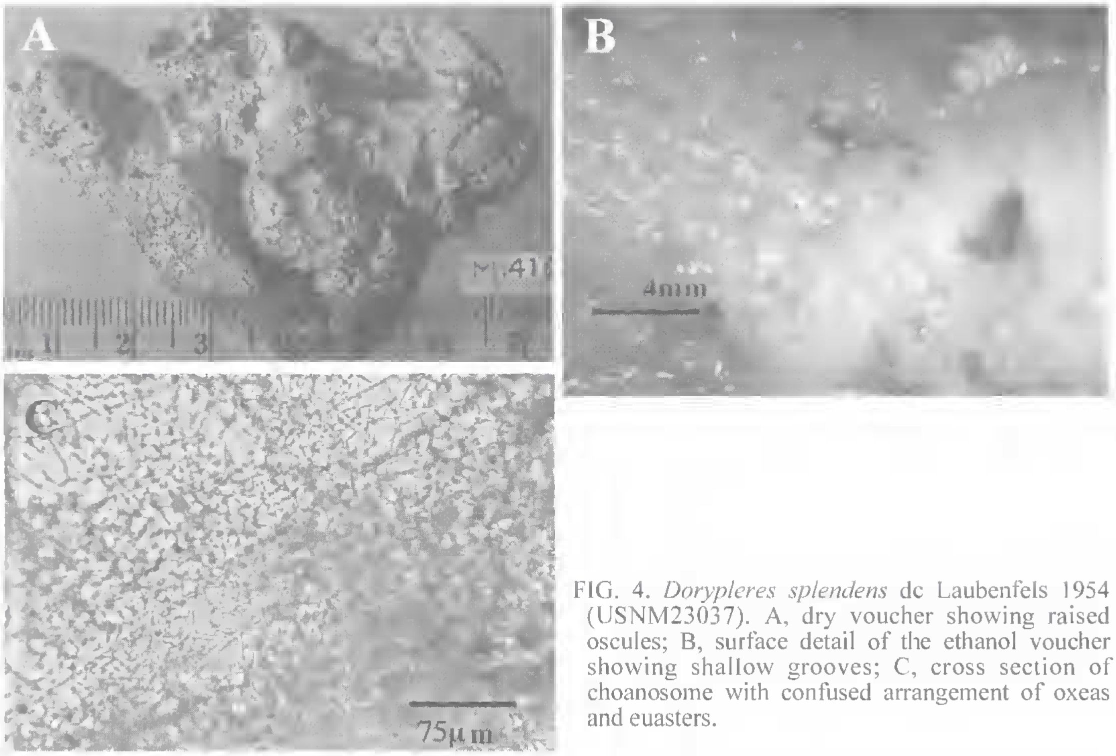

FIG. 4. *Dorypleres splendens* de Laubenfels 1954 (USNM23037). A, dry voucher showing raised oscules; B, surface detail of the ethanol voucher showing shallow grooves; C, cross section of choanosome with confused arrangement of oxeas and euasters.

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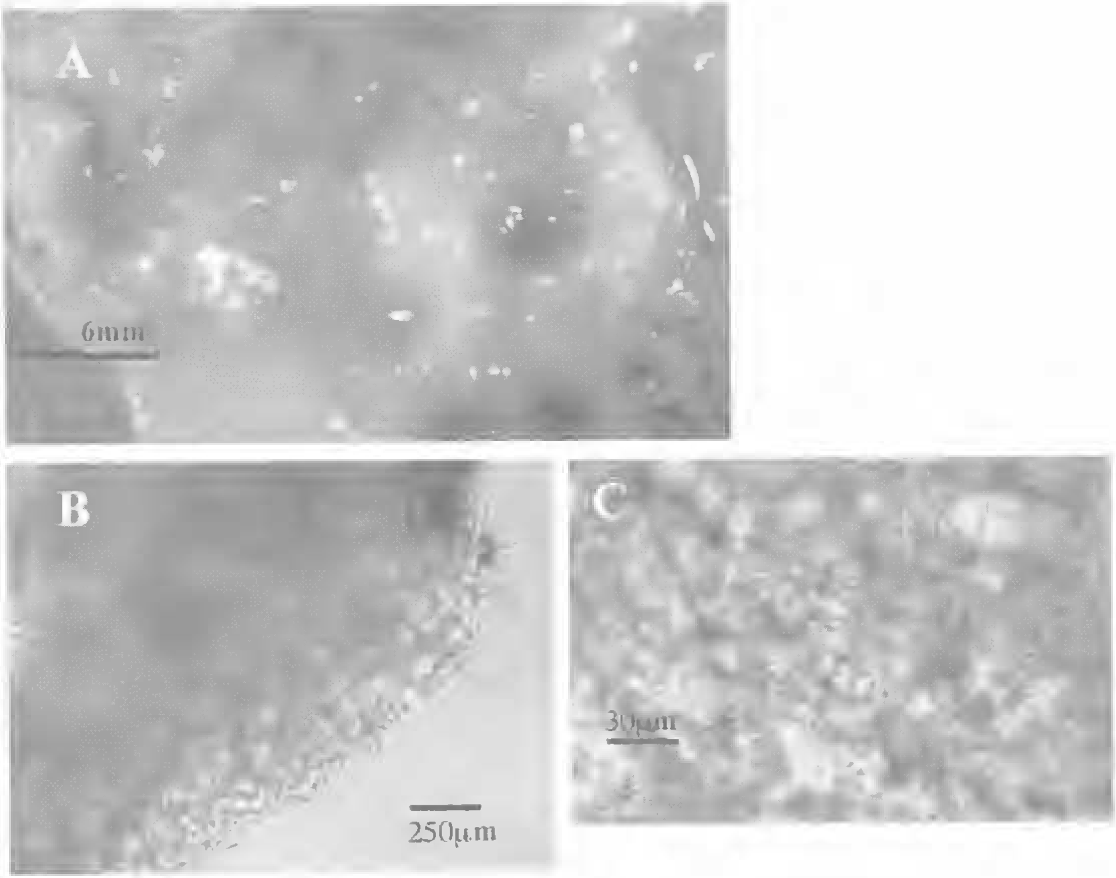

FIG. 5. *Zaplethea digonoxea* de Laubenfels 1950 (USNM22746). A, smooth surface of the ethanol voucher; B, cross section of the ectosome; C, cross section of the choanosome.

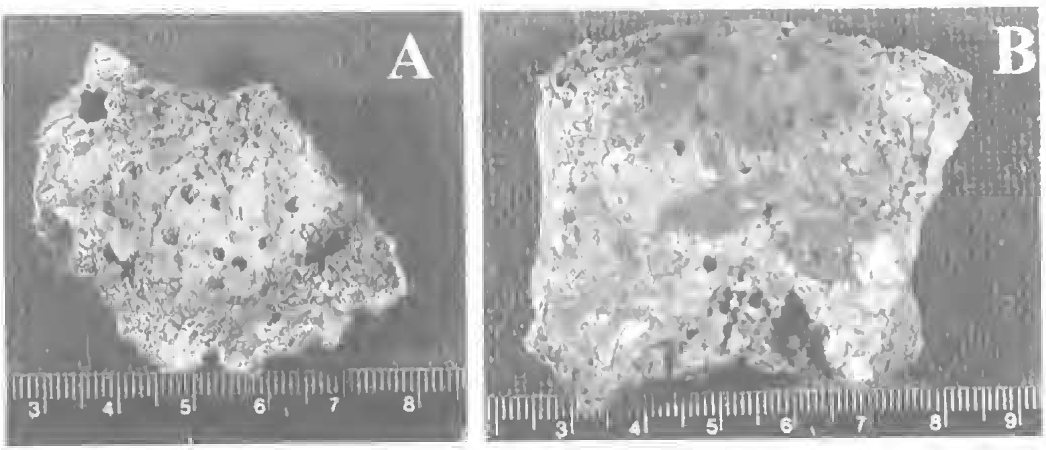

FIG. 6. *Vioa johnstoni* Schmidt 1862. A, dry voucher encrusting on coralline rock (LMJG15258/0); B, dry voucher encrusting on coralline rock (LMJG15256/0). Scales in cm.

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RECOVERY AND GROWTH OF THE GIANT BARREL SPONGE (*XESTOSPONGIA MUTA*) FOLLOWING PHYSICAL INJURY FROM A VESSEL GROUNDING IN THE FLORIDA KEYS. *Memoirs of the Queensland Museum 44: 532. 1999*:- On February 2, 1997, the 187m (614 feet) container ship 'Contship Houston' ran aground on the Florida reef tract near Maryland Shoal within the Florida Keys National Marine Sanctuary. This incident resulted in significant injury to coral reef resources over an area 650m (2,132 feet) in length. Hundreds of the Giant Barrel Sponge (*Xestospongia muta*) were damaged or destroyed as the ship approached the final grounding site, along with thousands of scleractinian corals and other reef organisms. A major coral reef restoration project is currently underway to address the physical and biological injury caused by the grounding. Over 3,000 broken and dislodged corals were reattached to the substrate within the inbound tract of the vessel, and large areas of rubble created by the ship's hull have been stabilised through a variety of techniques.

The purpose of this study was to assess the response of injured *Xestospongia* to the physical injury caused by the vessel grounding. As the vessel approached the grounding site, sponges which were in the path of the ship were subjected to various degrees of injury. This injury ranged from the minor breaking off of the tops of the sponges to the complete destruction of the sponge except for the basal tissue attached to the substrate. I located and marked 37 injured specimens with individual tags attached to plastic cable ties positioned tightly on the upper injured surface at two locations of each sponge. I monitored the sponges at two to three month intervals and measured upward linear growth from the cable ties. I also observed the condition and vitality of each sponge and the method by which the sponges responded to their injuries. All sponges were photographed at regular intervals.

During the course of the study, seven of the tagged sponges disappeared from the study site. Four of these were observed to have died from a wasting disease that was reported from numerous locations in the Florida Keys and the Caribbean. The causes of the disappearance of the other three were not directly observed. The 30 remaining sponges have survived and recovered from the direct physical injury at a minimum by healthy tissue regeneration of the damaged areas. The rate of upward linear growth ranged from zero to 4.48cm over 13 months, with an average upward linear growth of 1.42cm for all sponges. Eight specimens (27%) showed no upward growth over the observation period. The average growth rate for the sponges that did exhibit upward growth was 1.94cm. The most significant period of growth was in the late summer and throughout the fall, which corresponds to the period of warmest seawater temperatures. Upward linear growth was correlated with the degree of injury, with the moderate or slightly injured specimens growing at a faster rate than the badly injured ones. Of the four sponges that died from the wasting disease, three had been categorised as badly injured, which may suggest that injured sponges may be more susceptible to disease than non-injured sponges. ❒ *Porifera, Giant Barrel Sponge, Xestospongia muta, sponge growth rates, recovery from physical injury, coral reef injury and restoration, Florida Keys, Florida Keys National Marine Sanctuary.*

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